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(21) International Application Number: PCT/SE99/01913 (22) International Filing Date: 22 October 1999 (22.10.99) (30) Priority Data: 09/179,107 26 October 1998 (26.10.98) US (71) Applicant: LUNDAGRION AB [SE/SE]; c/o Bengt Bengtzell, Lunds Redovisningsbyrå, B & B HB, Box 1027, S-221 04 Lund (SE). (72) Inventor: ÖSTE TRIANTAFYLLOU, Angeliki; Kollegievägen 91, S-224 73 Lund (SE). (74) Agent: CONIMAR AB; Box 2086, S-141 02 Huddinge (SE).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: METHOD FOR THE ISOLATION OF A β -GLUCAN COMPOSITION FROM OATS AND PRODUCTS MADE THERE- FROM (57) Abstract A method for producing, from an oats flour fraction, a water soluble β -glucan composition having a high β -glucan/glucose weight ratio, preferably a ratio of 15:1 or more, comprises the use of β -amylase in an amount sufficient to transform more than 50 % by weight, preferably more than 65 % by weight, of the starch contained in the oats flour fraction to maltose. The enzymes pullulanase and/or protease may be used in combination with β -amylase. Also disclosed are corresponding compositions which may be further processed, as well as food products provided with them.		

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METHOD FOR THE ISOLATION OF A β -GLUCAN COMPOSITION FROM OATS
AND PRODUCTS MADE THEREFROM

FIELD OF THE INVENTION

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The present invention relates to a method for the isolation of a water soluble native β -glucan composition from oats, to the corresponding composition as such, and to products prepared from this composition.

10

BACKGROUND OF THE INVENTION

Water soluble native β -glucan is of major nutritional interest. It is the chemical constituent of 'soluble dietary fiber', SDF, considered to be responsible for the association between oats products and reduced risk for coronary heart disease. In this context the term 'native' indicates that the carbohydrate has not been degraded enzymatically to a substantial extent during its isolation. A variety of health food products rich in SDF are currently on the market. Barley and oats are rich in SDF. Oats SDF is documented as being particularly healthy.

A method for making a SDF composition from oats is disclosed in U.S. Patent No. 4,996,063 (Inglett). The method of Inglett comprises gelatinizing a milled oat substrate prior to treating it with an α -amylase which may yield substantial amounts of glucose. From the hydrolyzed mixture an aqueous SDF fraction is recovered by separating water insoluble material. The usefulness of the β -glucan product produced by the method of US 4,996,063 as a food additive is however diminished by its high glucose content. A high content of glucose promotes the formation of undesired, that is, colored and bitter products on heating in the presence of amino acids (Maillard

reaction). Moreover the Maillard reaction preferentially consumes lysine which is an amino acid essential to man. In many applications a high glucose content is a drawback because of the sweetness of glucose.

5

In the context of producing and further handling SDF it is important to prevent the action of β -glucanase possibly present to avoid β -glucan degradation which would ensue in loss of nutritional value. It is also important to provide
10 the β -glucan product essentially free of β -glucanase. The production of pure and stable SDF from oats is hampered by its rather high content of fat, proteins and, in particular, β -glucanase.

15 OBJECTS OF THE INVENTION

It is an object of the present invention to provide a method of the aforementioned kind enabling the production of a stable, high yield β -glucan composition from oats.

20

It is another object of the invention to provide method for the production of a stable, high yield, water soluble β -glucan composition from oats which has low sweetness and high temperature stability under conditions of food preparation and
25 food processing.

It is a further object of the present invention to provide corresponding compositions and products prepared from them.

30 SUMMARY OF THE INVENTION

According to the invention is disclosed a method for producing, from an oats flour fraction, a water soluble β -glucan composition having a high β -glucan/glucose weight

ratio, preferably a ratio of 15:1 or more, the method comprising the use of β -amylase in an amount sufficient to transform more than 50 % by weight, preferably more than 65 % by weight, of the starch contained in the oats flour fraction to maltose. In addition substantial amounts of water soluble oligosaccharides are formed.

It is preferred for the method according to the invention to comprise, in addition to the use of β -amylase, the use of pullulanase which 'promotes' the action of β -amylase. Pullulanase is a starch debranching enzyme which also aids saccharification to oligosaccharides and maltose as the dominating monosaccharide while not promoting the formation of glucose.

According to an advantageous aspect of the invention it is preferred for the method according to the invention to additionally comprise the use of protease. This is particularly advantageous if a purification of the β -glucan composition of the invention to increase its content of water soluble β -glucan is contemplated.

According to another advantageous aspect of the invention it is preferred for the method of the invention to comprise the use of (in terms of enzymatic activity in relation to β -amylase) of α -amylase in an amount capable to accelerate the starch degradation process but not to form substantial amounts of glucose. It is preferred to add from 0 to 10%, more preferred from 1 to 5%, of α -amylase in terms of enzymatic activity relative to β -amylase. The person skilled in the art will realize that the amount of α -amylase needed for this end will vary according to reaction conditions (time, temperature, etc.) but can be easily determined by simple experimentation.

In particular, the method of the invention comprises the following steps:

- selecting an oats variety rich in β -glucan and, optionally, low in fat;
- 5 - producing oats flour by dry-milling of said oats variety;
- selecting an oats flour fraction rich in β -glucan by sieving or other particle size/weight discriminating means;
- adding to an aqueous medium the flour fraction which had been heat-treated as such or prepared from a heat treated
- 10 oats variety or from heat-treated oats flour, β -amylase and, optionally, pullulanase;
- adding to an aqueous medium the thus treated flour fraction, β -amylase and, optionally, pullulanase;
- heating the thus produced suspension at a temperature above
- 15 30°C for a time period sufficient to substantially degrade starch;
- inactivating added enzymes by further heating of the suspension and/or by other means;
- forming a water soluble β -glucan composition by removing
- 20 water insoluble material;
- optionally, concentrating and/or drying said β -glucan composition.

It is preferred for the temperature at which the suspension is

25 heated to degrade most of the starch and proteins to be from 52 °C to 65 °C, in particular about 55°C.

The temperature at which the suspension is heated to inactivate the added enzymes is preferably from about 80°C to

30 about 95°C.

It is preferred to form the water soluble β -glucan composition by removing water insoluble material by centrifugation and/or filtration.

- 5 The β -glucan composition of the invention may be used in form of its aqueous solution which, at higher concentrations, turns into a gel at room temperature, or in form of a powder obtained by, for instance, freeze or spray drying of aqueous solutions of the β -glucan composition.

10

The β -glucan composition of the invention can be used as a food additive, for instance as an additive of soft drinks and beer, the latter use being particularly preferred.

- 15 According to an advantageous aspect the β -glucan composition of the invention can be treated with a protease, in particular alkalase®, to degrade proteins to peptides and amino acids. This is particularly advantageous if removal of low-molecular weight compounds, for instance of compounds having a molecular weight of below 200, is contemplated. Appropriate methods for removal of low-molecular weight constituents include ultra-filtration, reverse osmosis, and gel filtration. It is also within the scope of the invention to add such enzymes prior or during the formation of the β -glucan composition of the invention, for instance during the starch degradation step of the method of the invention.
- 20
- 25

- The present invention also discloses food products enriched with the β -glucan product of the invention. Enriched liquid products include fruit juices, beer, mash, milk and fermented liquid and semi-liquid dairy products, milk and cream substitutes, soft drinks, syrups, liquid honey, etc.
- 30

The β -glucan product of the invention may also be used as a gelling additive in various food products. The freeze dried product is particularly suited as additive to solid or essentially solid food products, like bread, biscuits, chips, etc.

Further advantages of the invention are disclosed in the claims and will also be evident from a preferred, not limiting embodiment of the invention described in the following in greater detail by reference to a single Figure showing a chart illustrating the process the invention.

Materials. A commercial heat-treated oats flour fraction high in β -glucan, 'HAVREMJÖL C45', was obtained from Skåne-möllan (Tågarp, Sweden). Oats flour fractions high in β -glucan can be also be obtained by applying the teaching of US 5,063,078 (Frohse) to oats. β -Amylase was obtained from Genencor International, Inc. (Rochester, NY, USA). α -Amylase, pullulanase and protease, for example Alcalase®, were obtained from Novo Nordisk, (Valby, Denmark).

Enzymatic degradation of starch and, optionally, protein.
To a thermostat-controlled, heat-mantled 100 l stainless steel tank 1 provided with an efficient stirrer containing 30 l of water at 55°C is added 54 g of β -amylase and 18 g of pullulanase. Then 6 kg of heat treated oat meal is added by a screw feeder 2 within 20 minutes so as to keep the viscosity below 128 mPas at a shear rate of 697 s⁻¹. The suspension is heated under stirring at 55 °C. The viscosity of the suspension is monitored by a Bohlin Visco 88 meter. The dry matter content of the suspension thus reaches about 20%. After 2 hrs the viscosity drops to 40 mPas at a shear rate of 697 s⁻¹. Then 5 g of Alcalase® is added and heating at 55°C is continued for another 30 minutes. Via a balance vessel 3 the

suspension is pumped to a steam injector 4 in which its temperature is raised to 90°C to inactivate added enzymes. From there the suspension is cooled, preferably to a temperature below 40°C, for instance by pumping it through a heat exchanger 5 in which it is brought to room temperature, and further to a decanter centrifuge 6 (5,000 rpm) for separation of remaining solids (at 13; about 1/3 by weight of solids at start) which may be used, for instance, for the production of animal foodstuff.

10

The clear solution thus obtained contains about 2% of native soluble β -glucan. The β -glucan solution is collected in a collection tank 7 from which it is discharged in portions and transported to a pasteurization station 8. After passing station 8 it is cooled to ambient temperature in a heat exchanger 9 and stored in a storage tank 10 from which it can removed for additional treatment, such as evaporation in an evaporator 11 to produce a highly viscous gel or freeze-drying to yield a porous powder containing 17 % by weight of β -glucan. Alternatively the pasteurized solution can be discharged from the storage tank 10 for packaging 12 and transport to other sites to be used as such. If desired the solution can be purified by removing low molecular constituents, mainly hydrolysis products of starch and proteins, by ultra-filtration.

25

C l a i m s

1. A method for producing, from an oats flour fraction, a water soluble β -glucan composition having a high β -glucan/glucose weight ratio, preferably a ratio of 15:1 or more, the method comprising the use of β -amylase in an amount sufficient to transform more than 50 % by weight, preferably more than 65 % by weight, of the starch contained in the oats flour fraction to maltose.
2. The method of claim 1, comprising the use of pullulanase and/or protease.
3. The method of claim 1 or 2, comprising the use of α -amylase in an amount so as to essentially avoid the formation of glucose, while promoting the action of β -amylase.
4. The method of claim 3, wherein the amount of α -amylase corresponds to from 0 to 10%, preferably from 1 to 5%, of the enzymatic activity of added β -amylase.
5. A method for producing of a water soluble β -glucan composition from oats which has a high β -glucan/glucose weight ratio, comprising the following steps:
 - selecting at least one member of the group consisting of (a) an oats variety rich in β -glucan and optionally low in fats, (b) an oats flour comprising said oats variety dry-milled and (c) a fraction of said oats flour rich in β -glucan;
 - inactivating carbohydrate degrading enzymes in said selected member;
 - dry milling said inactivated member;

- combining said dry milled member with an aqueous medium and β -amylase and, optionally, pullulanase, to form a suspension;
- heating the thus produced suspension at a temperature above
5 30°C for a time period sufficient to substantially degrade starch to oligosaccharides and maltose as the dominating disaccharide;
- inactivating said β -amylase and, when present, pullulanase enzymes;
- 10 - removing water insoluble material to form a water soluble β -glucan composition.

6. The method of claim 5, wherein the temperature at which the suspension is heated to degrade most of the starch and
15 proteins is from 52 °C to 65 °C, preferably of about 55°C.

7. The method of claim 5 or 6, wherein the temperature at which the suspension is heated to inactivate the added enzymes is from about 80 °C to about 95 °C.

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8. The method of any of claims 5-7, wherein the water soluble β -glucan composition is isolated by removing water insoluble material by centrifugation and/or filtration.

25 9. The method of any of claims 5-8, wherein the β -glucan composition is spray or freeze dried.

10. The method of any of claims 5-9, wherein low molecular weight compounds, such as amino acids and sugars, are removed
30 by ultra-filtration.

11. A freeze-dried water soluble β -glucan composition comprising 10% by weight or more of water soluble β -glucan from oats.
- 5 12. A water soluble β -glucan composition produced by the process of any of claims 1-10.
13. The composition of claim 12, wherein the content of water soluble β -glucan is 15% or more by weight.
- 10 14. A food product enriched with the β -glucan composition obtained by the process of any of claims 1-10.
- 15 15. A food product enriched with the β -glucan composition of any of claims 11-13.
16. Beverages, such as soft drinks and beer, enriched with the β -glucan composition obtained by the process of any of claims 1-10.
- 20 17. Beer or mash enriched with the β -glucan composition of any of claims 11-13.
- 25 18. A β -glucan product obtained by treatment of the β -glucan composition of any of claims 11-13 with an enzyme promoting the degradation of poly- and oligosaccharides to maltose, such as pullulanase, and/or with a protease, such as alkalase®, to degrade proteins to peptides and amino acids.
- 30 19. The product of claim 18 purified by one or several of reverse osmosis, ultra-filtration and gel filtration to remove constituents having a molecular weight below 200.

20. The product of claim 18, wherein the enzyme promoting the degradation of poly- and oligosaccharides to maltose consists of pullulanase.

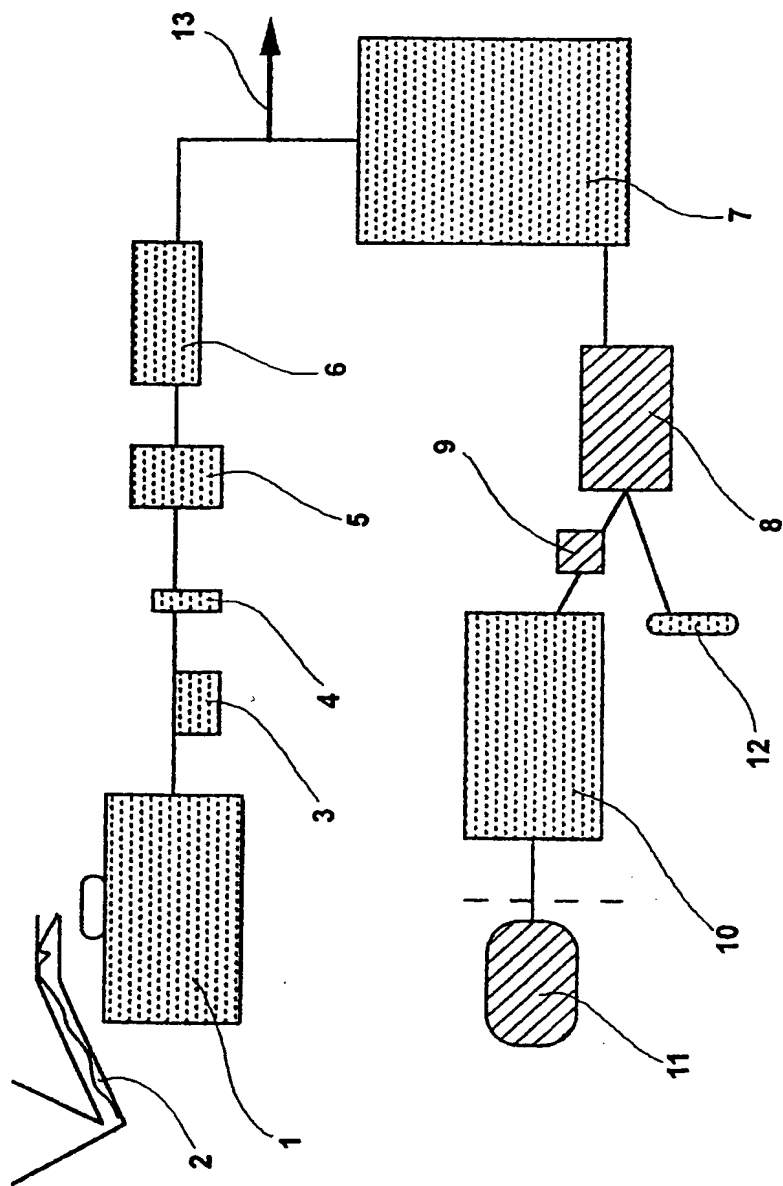


Figure 1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 99/01913

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A23L 1/105, C08B 37/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9507628 A1 (LINDAHL, L. ET AL), 23 March 1995 (23.03.95) --	1-20
X	US 5616355 A (W.E. HAAST ET AL), 1 April 1997 (01.04.97), abstract, claims --	9,11,15, 17-20
A	US 4996063 A (G.F. INGLETT), 26 February 1991 (26.02.91) --	1-20
A	EP 0231729 A1 (BERGKVIST, R.R. ET AL), 12 August 1987 (12.08.87), page 2, line 24 - page 3, line 29 --	1-20

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

27 January 2000

Date of mailing of the international search report

2000-02-17

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 99/01913

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9428742 A1 (EXAVENA OY), 22 December 1994 (22.12.94), claim 5, abstract -- -----	2,18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE99/01913

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE99/01913

According to Article 34 (3) (a-c) and Rule 13.2, an international application shall relate to one invention only or to a group of inventions linked by one or more of the same or corresponding "special technical features", i.e. features that define a contribution which each of the inventions makes over the prior art. Two separate groups of inventions were found in the present application, namely:

1. A method for production of a water soluble β -glucan composition, according to claims 1-10 (completely), and uses of that composition, according to claims 12-14 and 16 (completely) and 15 and 17-20 (partially).
2. A water soluble β -glucan composition, according to claim 11, and uses of the composition, according to claims 15 and 17-20 (all partially).

The technical features of invention 2 are freeze-drying and level of β -glucan content. These technical features are absent from the inventions of group 1. The two groups of inventions are thus not linked by a special technical feature as required by PCT Rule 13.2. However, as the additional effort of searching invention 2 did not justify an additional search fee, the entire application has been searched.

INTERNATIONAL SEARCH REPORT
Information on patent family members

02/12/99

International application No.
PCT/SE 99/01913

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